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Nucleotide Sequence of wtGFP (Chalfie et al, Science, (1994), 263, 802-5): SEQ ID NO:1

atg agt aaa gga gaa gta ctt ttc act gga gtt gtc cca att ctt gtt 48 gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc agt gga gag 96 ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt att tgc 144 act act gga aaa cta cct gtt cca tgg cca aca ctt gtc act act ttc 192 tct tat ggt gtt caa tgc ttt tca aga tac cca gat cat atg aaa cgg 240 cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa aga 288 act ata ttt ttc aaa gat gac ggg aac tac aag aca cgt gct gaa gtc 336 aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa ggt att 384 gat ttt aaa gaa gat gga aac att ctt gga cac aaa ttg gaa tac aac 432 tat aac tca cac aat gta tac atc atg gca gac aaa caa aag aat gga 480 atc aaa gtt aac ttc aaa att aga cac aac att gaa gat gga agc gtt 528 caa cta gca gac cat tat caa caa aat act cca att ggc gat ggc cct 576 gtc ctt tta cca gac aac cat tac ctg tcc aca caa tct gcc ctt tcg 624 672 aaa gat ccc aac gaa aag aga gac cac atg gtc ctt ctt gag ttt gta aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa tag 717

FIGURE 1

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Amino Acid Sequence of wtGFP (Chalfie et al, Science, (1994), 263, 802-5 SEQ ID NO:2

Met 1	Ser	Lys	Gly	Glu 5	Glu	Leu	Phe	Thr	Gly 10	Val	Val	Pro	Ile	Leu 15	Val
Glu	Leu	Asp	Gly 20	Asp	Val	Asn	Gly	His 25	Lys	Phe	Ser	Val	Ser 30	Gly	Glu
Gly	Glu	Gly 35	Asp	Ala	Thr	Tyr	Gly 40	Lys	Leu	Thr	Leu	Lys 45	Phe	Ile	Cys
Thr	Thr 50	Gly	Lys	Leu	Pro	Val 55	Pro	Trp	Pro	Thr	Leu 60	Val	Thr	Thr	Phe
Ser 65	Tyr	Gly	Val	Gln	Cys 70	Phe	Ser	Arg	Tyr	Pro 75	Asp	His	Met	Lys	Arg 80
His	Asp	Phe	Phe	Lys 85	Ser	Ala	Met	Pro	Glu 90	Gly	Tyr	Val	Gln	Glu 95	Arg
Thr	Ile	Phe	Phe 100	Lys	Asp	Asp	Gly	Asn 105	Tyr	Lys	Thr	Arg	Ala 110	Glu	Val
Lys	Phe	Glu 115	Gly	Asp	Thr	Leu	Val 120	Asn	Arg	Ile	Glu	Leu 125	Lys	Gly	Ile
Asp	Phe 130	Lys	Glu	Asp	Gly	Asn 135	Ile	Leu	Gly	His	Lys 140	Leu	Glu	Tyr	Asn
Tyr 145	Asn	Ser	His	Asn	Val 150	Tyr	Ile	Met	Ala	Asp 155	Lys	Gln	Lys	Asn	Gly 160
Ile	Lys	Val	Asn	Phe 165	Lys	Ile	Arg	His	Asn 170	Ile	Glu	Asp	Gly	Ser 175	Val
Gln	Leu	Ala	Asp 180	His	Tyr	Gln	Gln	Asn 185	Thr	Pro	Ile	Gly	Asp 190	Gly	Pro
Val	Leu	Leu 195	Pro	Asp	Asn	His	Tyr 200	Leu	Ser	Thr	Gln	Ser 205	Ala	Leu	Ser
Lys	Asp 210	Pro	Asn	Glu	Lys	Arg 215	Asp	His	Met	Val	Leu 220	Leu	Glu	Phe	Val
Thr 225	Ala	Ala	Gly	Ile	Thr 230	His	Gly	Met	Asp	Glu 235	Leu	Tyr	Lys		

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<u>Predicted Amino Acid Sequence of F64L-S175G-E222G-GFP:</u> <u>SEQ ID NO:3</u>

Met 1	Ser	Lys	Gly	Glu 5	Glu	Leu	Phe	Thr	Gly 10	Val	Val	Pro	Ile	Leu 15	Val
Glu	Leu	Asp	Gly 20	Asp	Val	Asn	Gly	His 25	Lys	Phe	Ser	Val	Ser 30	Gly	Glu
Gly	Glu	Gly 35	Asp	Ala	Thr	Tyr	Gly 40	Lys	Leu	Thr	Leu	Lys 45	Phe	Ile	Cys
Thr	Thr 50	Gly	Lys	Leu	Pro	Val 55	Pro	Trp	Pro	Thr	Leu 60	Val	Thr	Thr	Leu
Ser 65	Tyr	Gly	Val	Gln	Cys 70	Phe	Ser	Arg	Tyr	Pro 75	Asp	His	Met	Lys	Arg 80
His	Asp	Phe	Phe	Lys 85	Ser	Ala	Met	Pro	Glu 90	Gly	Tyr	Val	Gln	Glu 95	Arg
Thr	Ile	Phe	Phe 100	Lys	Asp	Asp	Gly	Asn 105	Tyr	Lys	Thr	Arg	Ala 110	Glu	Val
Lys	Phe	Glu	Gly 115	Asp	Thr	Leu	Val 120	Asn	Arg	Ile	Glu	Leu 125	Lys	Gly	Ile
Asp	Phe 130	Lys	Glu	Asp	Gly	Asn 135	Ile	Leu	Gly	His	Lys 140	Leu	Glu	Tyr	Asn
Tyr 145	Asn	Ser	His	Asn	Val 150	Tyr	Ile	Met	Ala	Asp 155	Lys	Gln	Lys	Asn	Gly 160
Ile	Lys	Val	Asn	Phe 165	Lys	Ile	Arg	His	Asn 170	Ile	Glu	Asp	Gly	Gly 175	Val
Gln	Leu	Ala	Asp 180	His	Tyr	Gln	Gln	Asn 185	Thr	Pro	Ile	Gly	Asp 190	Gly	Pro
Val	Leu	Leu 195	Pro	Asp	Asn	His	Tyr 200	Leu	Ser	Thr	Gln	Ser 205	Ala	Leu	Ser
Lys	Asp 210	Pro	Asn	Glu	Lys	Arg 215	Asp	His	Met	Val	Leu 220	Leu	Gly	Phe	Val
Thr 225	Ala	Ala	Gly	Ile 230	Thr	His	Gly	Met	Asp	Glu 235	Leu	Tyr	Lys		

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<u>Predicted Amino Acid Sequence of F64L-S65T-S175G-GFP:</u> <u>SEQ ID NO:4</u>

Met 1	Ser	Lys	Gly	Glu 5	Glu	Leu	Phe	Thr	Gly 10	Val	Val	Pro	Ile	Leu 15	Val
Glu	Leu	Asp	Gly 20	Asp	Val	Asn	Gly	His 25	Lys	Phe	Ser	Val	Ser 30	Gly	Glu
Gly	Glu	Gly 35	Asp	Ala	Thr	Tyr	Gly 40	Lys	Leu	Thr	Leu	Lys 45	Phe	Ile	Cys
Thr	Thr 50	Gly	Lys	Leu	Pro	Val 55	Pro	Trp	Pro	Thr	Leu 60	Val	Thr	Thr	Leu
Thr 65	Tyr	Gly	Val	Gln	Cys 70	Phe	Ser	Arg	Tyr	Pro 75	Asp	His	Met	Lys	Arg 80
His	Asp	Phe	Phe ·	Lys 85	Ser	Ala	Met	Pro	Glu 90	Gly	Tyr	Val	Gln	Glu 95	Arg
Thr	Ile	Phe	Phe 100	Lys	Asp	Asp	Gly	Asn 105	Tyr	Lys	Thr	Arg	Ala 110	Glu	Val
Lys	Phe	Glu 115	Gly	Asp	Thr	Leu	Val 120	Asn	Arg	Ile	Glu	Leu 125	Lys	Gly	Ile
Asp	Phe 130	Lys	Glu	Asp	Gly	Asn 135	Ile	Leu	Gly	His	Lys 140	Leu	Glu	Tyr	Asn
Tyr 145	Asn	Ser	His	Asn	Val 150	Tyr	Ile	Met	Alá	Asp 155	Lys	Gln	Lys	Asn	Gly 160
Ile	Lys	Val	Asn	Phe 165	Lys	Ile	Arg	His	Asn 170	Ile	Glu	Asp	Gly	Gly 175	Val
Gln	Leu	Ala	Asp 180	His	Tyr	Gln	Gln	Asn 185	Thr	Pro	Ile	Gly	Asp 190	Gly	Pro
Val	Leu	Leu 195	Pro	Asp	Asn	His	Tyr 200	Leu	Ser	Thr	Gln	Ser 205	Ala	Leu	Ser
Lys	Asp 210	Pro	Asn	Glu	Lys	Arg 215	Asp	His	Met	Val	Leu 220	Leu	Glu	Phe	Val
Thr 225	Ala	Ala	Gly	Ile	Thr 230	His	Gly	Met	Asp	Glu 235	Leu	Tyr	Lys		

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Flow cytometry of GFP mutations

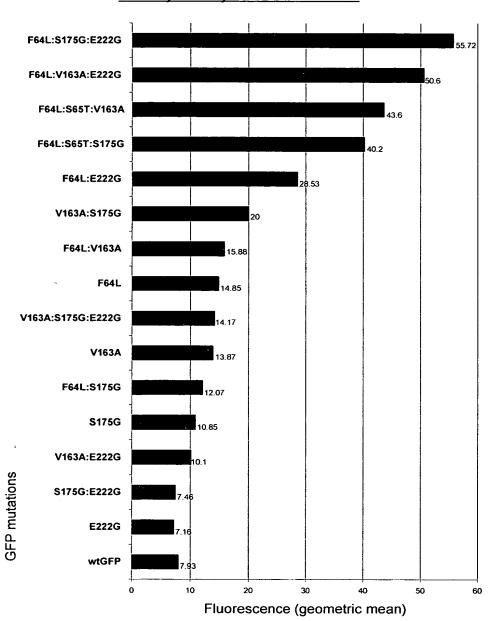


FIGURE 5

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Photobleaching of GFP mutations

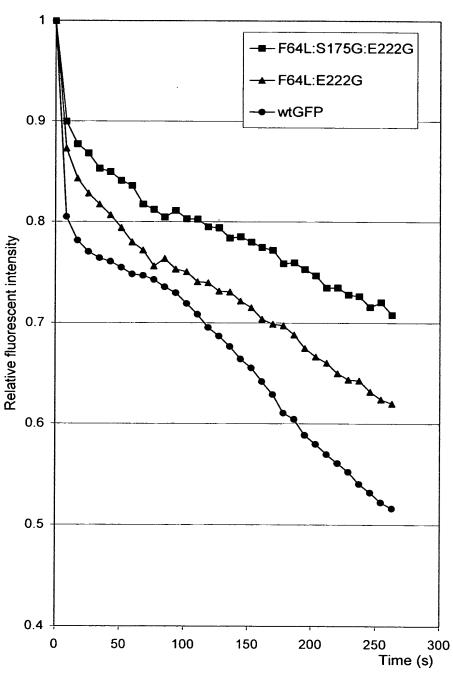


FIGURE 6

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<u>Live Cell CHO-HIR NFκB Assay</u> P65-tri GFP Assay T = 30 mins

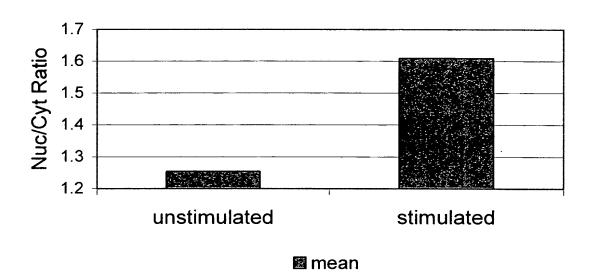


FIGURE 7

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